# **Bioturbation by the Invasive Rusty Crayfish (*Orconectes rusticus*) Affects Turbidity and Nutrients: Implications for Harmful Algal Blooms**

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## Abstract

Eutrophication and harmful algal blooms (HABs) are serious anthropogenic stressors impacting water quality and aquatic ecosystems worldwide. Although anthropogenic nutrient loading is a primary factor driving the rise in HABs, aquatic bioturbators may also contribute to the resuspension of nutrients and sediment into the water column and exacerbate HABs. Bioturbators are benthic organisms that rework bottom sediments in aquatic ecosystems through their daily activities, and can contribute to HABS by stirring up and resuspending nutrients and cyanobacteria cells. The rusty crayfish (*Orconectes rusticus*) is one such freshwater bioturbator that has established itself as an invasive species in central Ohio. The objective of this study was to examine the effect of crayfish density (low, high and no crayfish control) on turbidity and nutrient concentrations in a controlled laboratory experiment. In each of the three treatments, turbidity, nitrogen and phosphorus measurements were taken after a 24 hour acclimation period. Results indicate that the presence of crayfish significantly increased turbidity in the water column relative to the no crayfish control. Additionally, the concentration of nitrogen was significantly higher in the high density crayfish treatment and in the treatments with sediment. Opposite to predictions, phosphorous was higher in treatments without sediment and decreased in the presence of crayfish. Together, this suggests that through its daily activities, *O. rusticus* is causing a marked resuspension of sediments in the water column. This implies that through its role as a bioturbator, *O. rusticus* may indeed be exacerbating algae growth by agitating previously-settled nutrients that can further feed the growth of HABs, although more research would have to be completed on a larger scale to determine if they would indeed have a large enough effect to be biologically significant.

## Introduction

The rate of eutrophication (i.e., excessive growth of plants and algae) in freshwater systems has rapidly accelerated as a result of anthropogenic point and non-point sources of nutrients such as nitrogen and phosphorus. Ecologically, anthropogenic eutrophication can have multiple impacts on freshwater ecosystems by reducing dissolved oxygen and causing seasonal hypoxia, tainting drinking water, degrading recreational opportunities and causing blooms of algae and cyanobacteria (Arend et al. 2001) (Dodds et al. 2009). Economically, managing and mitigating these symptoms is estimated to cost the U.S. $2.2 billion annually (Dodds et al. 2009). Thus, anthropogenic eutrophication is both a nuisance, an economic cost and an environmental and health hazard in many aquatic ecosystems today.

Harmful algal blooms (HABs), in particular, are a particular threat to aquatic ecosystems worldwide and are observed to intensify as a result of eutrophication. HABs are actually not algae, but rather cyanobacteria that can produce certain cyanotoxins that can lead to very serious health problems and even death in fish, birds, and mammals (Diaz and Rosenberg 2008) (Zingone and Enevoldsen 2000). This generally toxic and unsightly cyanobacteria uses the excess nutrients (e.g., nitrogen (N) and phosphorus (P)) from nutrient loading into the water to fuel their spread and growth (Dolman et al. 2012). This increases the deposition of organic matter into the sediments as the excess cyanobacteria die off and sink to the bottom of the water column. As a consequence, the decomposition of excess cyanobacteria depletes oxygen levels in the water, often causing hypoxic conditions which can be detrimental to fish and aquatic macroinvertebrates. (Diaz and Rosenberg 2008).

Although anthropogenic nutrient loading is a primary factor driving the rise in HABs, aquatic bioturbators may also contribute to the resuspension of nutrients and sediment into the water column and exacerbate HABs. Benthic organisms can function as bioturbators that rework bottom sediments in aquatic ecosystems through their daily activities. For example, in the Baltic Sea, it has been identified that redistribution of organic contaminants is common in the upper portion of benthic sediment. Redistribution is accomplished primarily by the process of bioturbation by diving ducks, fish, and invertebrates. This redistribution adds concentrations of contaminants back into the water column in significant quantities. (Skei et al. 2000).

Bioturbators might also affect ecosystems by redistributing actual algae and cyanobacteria cells into the water column that have settled into the sediments, and as a result, contribute to HABs. One study found that where bioturbators were present in the studied ecosystem, recruitment of algae increased significantly (Ståhl-Delbanco and Hansson 2002). Crayfish specifically are extremely effective bioturbators because of their large size and their varying behavioral movements (Statzner 2012).

Freshwater crayfish can be classified into two groups: borrowing and non-burrowing. Non-burrowing crayfish will disturb sediments simply by walking, especially with their back pairs of walking legs. In addition, they exert a relatively strong force over a small area of sediment when they are startled by flipping their tail to propel them backward. They will also slow themselves down during walking by pressing their tails and claws into the sediment. Burrowing crayfish will also add to sediment disturbance by digging holes in the sediment (Statzner 2012). The rusty crayfish (*Orconectes rusticus*) is one such freshwater bioturbator that has established itself as an invasive species in central Ohio (Figure 1).

The objective of this study was to examine the effect of crayfish density (low, high and no crayfish control) on turbidity and nutrient concentrations in a controlled laboratory experiment. I hypothesized that in the treatments with high levels of crayfish, turbidity and nutrient concentrations would be higher than in those treatments with low or no levels of crayfish.

**Methodology**

Five-gallon plastic aquaria served as artificial habitats for *O. rusticus* in a 3 x 2 factorial design, manipulating crayfish density and presence of sediment. Treatments included: a high density (3 crayfish), with sediment; a low density (1 crayfish), with sediment; a control (0 crayfish), with sediment; a high density (3 crayfish), without sediment; a low density (1 crayfish), without sediment; a control (0 crayfish), without sediment.

The experiment was conducted in a greenhouse under natural photoperiod conditions and controlled temperature range of 65-75-degrees F. Sediment used in the experiment was collected from the Olentangy River Wetland Research Park and thoroughly homogenized prior to being added to the tanks. *O. rusticus* specimens were collected using kick seine nets from Big Walnut Creek at Ruffner Park in Galena, Ohio.

The plastic tubs were set up in the greenhouse with approximately 880 mL of sediment in treatments with sediment. Oxygenating bubblers were place in all tubs. Five gallons of ordinary tap water treated with water conditioner to remove heavy metals and chlorine (i.e, to make it safe for crayfish) was added to each tub. The experiment was set up in three blocks, where each block was run on a separate day. Three replicates of each treatment combination were included in each block. Therefore, each treatment combination was replicated nine times (n = 9/treatment, n = 54 total). After the sediment was added to the tubs, 24 hours were allowed to pass so that the sediment could settle after addition. After this initial 24 hours, turbidity readings were taken in each tub using a turbidimeter prior to the addition of crayfish to treatments with crayfish.

Once crayfish were added to their respective treatments, the experiment ran for another 24 hours. Crayfish were controlled for size and had a mean carapace length of 25mm, +/- 3mm. After 24 hours, turbidity was measured a second time using a turbidimeter in order to get the percent change in turbidity after the crayfish addition. Additionally, temperature, pH and dissolved oxygen were measured across all tanks using a YSI multimeter probe. Then, 100 mL water samples were taken from each tub to be analyzed in the laboratory for concentration of nitrogen and phosphorus.

The methods used to determine the concentration of nitrogen and phosphorus in each sample followed the protocols outlined by HACH. In order to calculate phosphorus, standard known concentrations of phosphate solutions were made and ran along with the samples in order to generate a standard curve. The method used an acid persulfate digestion using HACH's PhosVer3 reagent packets in order to convert all of the phosphorus to orthophosphate (HACH Method 8190). After the reaction was completed, the samples were read at 880 nm on a spectrophotometer and plotted on the standard curve in order to determine the unknown concentration (HACH Method 8048).

A similar method was used in order to determine nitrate-nitrogen concentration in all of the samples. Known standards of nitrate concentrations were also made in order to generate a standard curve. The protocol outlined by HACH for using NitraVer5 reagent packets was used (HACH Method 8039). The samples were read at 400 nm on the spectrophotometer upon completion of the reaction and plotted on the standard curve in order to determine the unknown concentration.

An analysis of variance (ANOVA) was run to test for the effect of crayfish, sediment, an interaction between crayfish and sediment, and block on 1) percent change in turbidity, 2) concentration of nitrogen (mg/L) and 3) concentration of phosphorus (mg/L). Model residuals were visually examined in order to check for a violation of the assumption of normality. Upon examination, the residuals were generally normally distributed. Although the residuals were normally distributed, the effect of outliers in the dataset was explored by removing them and re-running the ANOVAs. Results were the same. Therefore, in order to maintain a balanced design, the outliers were kept in the dataset.

**Results**

Results of the ANOVA indicated that there was a significant effect of crayfish density on the percent change in turbidity (Figure 2, Table 1). Specifically, turbidity was significantly higher in the high density crayfish treatment relative to the low density and no crayfish control treatments (ANOVA, $p = 0.0011$). There was no significant effect of the blocking factor, sediment, or an interaction between sediment and crayfish.

As predicted, there was a significant effect of sediment on nitrogen concentrations (Figure 3, Table 1). Specifically, nitrogen concentrations were significantly higher in treatments with sediment

(ANOVA, $p < 0.0001$). Additionally, there was a significant block effect on the concentration of nitrogen (ANOVA, $p = 0.0005$). There was a trend towards treatments with high crayfish density having a higher nitrogen concentration in comparison to the low density crayfish treatment and no crayfish control, however, this was no statistically significant (ANOVA, $p = 0.06$). There was no significant interaction between crayfish and sediment on nitrogen concentration.

Opposite to predictions, phosphorous was significantly higher in treatments without sediment and significantly decreased in the presence of crayfish (Figure 4, Table 1). Sediment did indeed have a significant effect on phosphorus concentration (ANOVA, $p < 0.0001$). There was no significant block effect or an interaction between crayfish and sediment on phosphorus.

Unexpectedly, pH was found to be significantly different in treatments with and without sediment (ANOVA, $p = 0.0428$). Additionally, there was a significant block effect on pH (ANOVA, $p = 0.0055$).

Water temperature and dissolved oxygen were analyzed and were found to be not statistically different across treatments over the duration of the experiment (Table 2). Mean water temperature was $23.60 \pm 0.11$°C and mean dissolved oxygen was $83.73 \pm 2.39$ % saturation.

**Discussion**

*Percent Change in Turbidity*

Results suggest that through its daily activities, *O. rusticus* is causing an increase in turbidity in the water column. However, interestingly, turbidity increased with increasing crayfish density regardless of whether treatments contained sediment. Increases in turbidity in the no sediment treatments may have been due to growth of algae within the experimental tanks during the course of the study. In separate tanks that were not part of the experiment, but contained crayfish, blooms of green algae would occur every 2-3 days. These algae could have been present in the water used in the experiment or attached to the crayfish themselves. One reason why turbidity may have been higher in the treatments with crayfish, but no sediment, is because crayfish excretion might have added nutrients to the water, which triggered the growth of algae. Although turbidity was higher in the low crayfish, sediment treatment in contrast to the control, it was lower than in the treatment without sediment. The difference between the sediment treatments in the low crayfish density treatment suggests that crayfish do have an effect on turbidity through the redistribution of sediments. Throughout the course of the experiment, crayfish were observed to exhibit movement to a significant degree that would imply that they are indeed contributing to sediment redistribution, especially directly after feeding periods. This backs up the information discussed previously regarding the high degree to which crayfish act as effective bioturbators that disturb sediment to a significant degree (Statzner 2012). However, the difference in turbidity between the treatments with and without sediment disappeared in the high density crayfish treatment. This could again be explained by the fact that crayfish excretion may have contained enough nutrients to feed algae growth in the tubs to a high enough degree that turbidity increased as a result of the high density of crayfish rather than presence or absence of sediment.

*Concentration of Nitrate-Nitrogen*

As predicted, there was a significant effect of sediment on nitrogen in the water. Treatments with sediment have significantly higher nitrogen concentrations than treatments without sediment. Tanks with both sediment and a high density of crayfish had higher nitrogen concentrations in comparison to the tanks with sediment and no crayfish. This suggests that crayfish activity might contribute to the increase in nitrogen in the water column. Again, the qualitative observations that the crayfish exhibited a significant degree of movement throughout the course of the study back up the suggestion that the role of crayfish as bioturbators does indeed contribute to resuspension of nitrogen.

It is also possible that the higher nitrogen levels in tanks with sediment and a high density of crayfish were due to the crayfish excretion, as well. Although there was not a significant effect of crayfish on nitrogen concentration, there was a trend towards nitrogen increasing as crayfish density increased (Figure 3). A previous study found that crayfish do excrete nitrate and ammonia in sizable amounts to the benthic environment (Flint 1975). Using this information, it is important to consider that some of the nitrogen measured in this experiment could have been excreted from the crayfish. Additionally, the significant effect of blocks on nitrogen concentrations might further explain this phenomenon. With each new block of the three blocks, the concentration of nitrogen was observed to be higher than the previous. This might be explained by the fact that the crayfish may have been stressed by the end of the experiment and may have been excreting more as a result.

*Concentration of Orthophosphate-Phosphorus*

Unexpectedly, phosphorus concentrations decreased in the presence of sediment and crayfish. The highest levels of phosphorus observed were in the no crayfish control with no sediment (Figure 4). In other words, these tubs had nothing in them except for tap water treated with water conditioner. Conversely, the lowest levels of phosphorus were in the tubs with high levels of crayfish and sediment. This outcome was opposite to predictions and suggests that sediment might actually lock up excess phosphorus from the water column. It has been observed that phosphorus accumulates in and binds to aquatic benthic sediments. Oxic conditions in the benthic zone favor chemical processes that cause phosphate, a highly reactive anion, to be sorbed to the organic material in the sediment. Additionally, bioturbation and the presence of organisms in the sediment can increase oxic conditions due to their stirring and mixing behaviors. (Søndergaard 2003). Thus, the crayfish may have been aerating the sediment in the tubs to increase the adherence of phosphorus to the sediments through their normal daily movements.

*pH*

Unexpectedly, pH varied across different blocks and between the tubs with and without sediment. This might be explained by the fact that for each different block, the water in the tubs was replaced with new water. There was probably a small degree of variation in the pH of the tap water used which, although normal, caused the significant difference across blocks. Additionally, although the sediment used was homogenized prior to its use in the study, it is possible that there were still slight differences in the pH across all of the sediment which may have contributed to the significant difference in pH between tubs with and without sediment.

*Challenges and Further Research*

Potential modifications to narrow down some of the uncertainties discussed previously include gently washing the bodies of any specimens used in a study such as this one to ensure algae cell removal prior to their use in the experiment. This would to reduce potential bias from contamination of outside sources of algae blooms and turbidity. Additionally, the sediment used in a study such as this should be analyzed for the concentration of nitrogen and phosphorus already present in the sediment. This will allow the nutrient concentrations in the sediment to be accounted for when analyzing the concentrations of nutrients in the water column at the close of the experiment.

Data from this study implies that through its role as a bioturbator, *O. rusticus* may have an effect on algae growth by agitating previously settled nutrients that can further feed the growth of HABs, but more research would have to be completed on a larger scale to determine if they would indeed have a large enough effect to be biologically significant. In a larger setting, such as a lake, the activity by the crayfish may not be significant enough to reach the the surface or near surface of the water column to have a significant effect on turbidity or nutrient concentrations. Completing a study such as this one on a larger scale in a natural setting will help to answer some of these questions.

**Acknowledgements**

I would like to thank Dr. Lauren Pintor for advising me on this study. I would also like to thank Lauren Hostert for assisting with sample analysis and Chris Johnson for assisting with crayfish collection.

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**Tables and Figures**

Figure 1. Distribution of *O. rusticus* by USGS drainages, showing native and introduced population ranges. (http://nas.er.usgs.gov/taxgroup/crustaceans/crayfish.html)

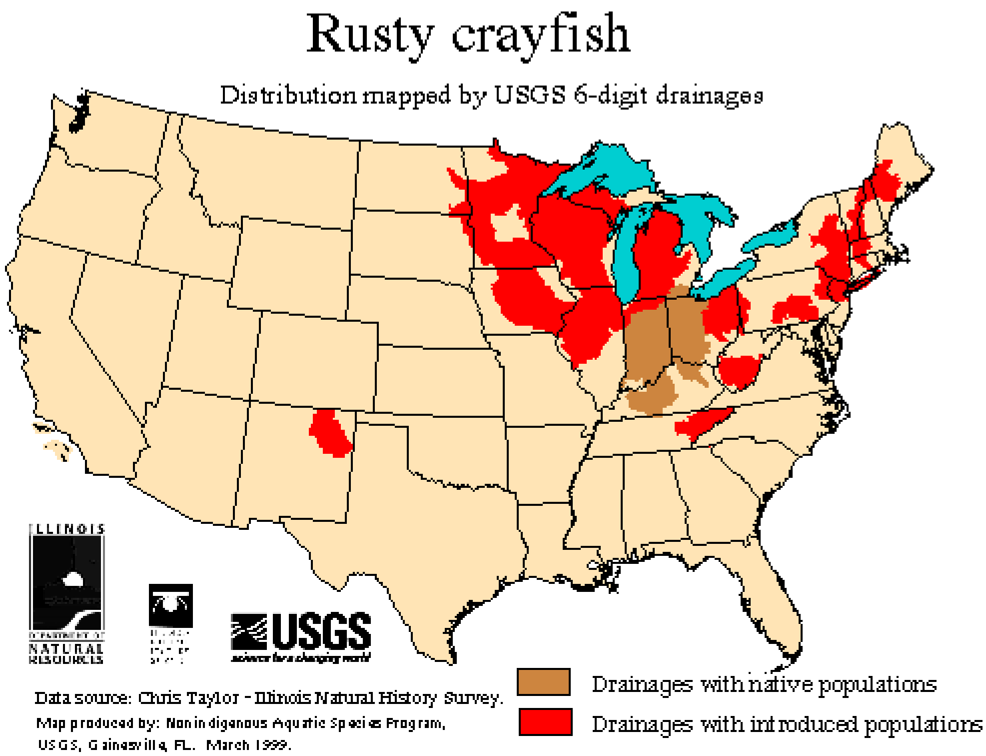


Table 1. F and p values across treatments in terms of percent change in turbidity, concentration of nitrogen, concentration of phosphorus, and pH.

| | **% Change Turbidity** | **N** | **P** | **pH** |
|---|---|---|---|---|
| **Crayfish** | F2,44 = 7.93<br>p = 0.001* | F2,44 = 2.98<br>p = 0.06 | F2,44 = 3.93<br>p = 0.03 | F2,44 = 3.06<br>p = 0.06 |
| **Sediment** | F1,44 = 0.46<br>p = 0.50 | F1,44 = 97.66<br>p < 0.0001* | F1,44 = 67.24<br>p < 0.0001* | F1,44 = 4.34<br>p < 0.0428* |
| **Crayfish x Sediment** | F2,44 = 0.50<br>p = 0.61 | F2,44 = 0.05<br>p = 0.95 | F2,44 = 0.15<br>p = 0.86 | F2,44 = 0.77<br>p = 0.47 |
| **Block** | F2, 44 = 2.40<br>p = 0.10 | F2,44 = 8.97<br>p = 0.0005* | F2, 44 = 2.95<br>p = 0.06 | F2, 44 = 5.84<br>p = 0.0055* |

Table 2. Means and standard errors across blocks for temperature, pH, and DO.

| Variable | Block 1 | Block 2 | Block 3 | All Blocks |
|---|---|---|---|---|
| Temperature (°C) | 22.93 ± 0.08 | 24.52 ± 0.11 | 23.35 ± 0.09 | 23.60 ± 0.11 |
| pH | 7.74 ± 0.08 | 8.04 ± 0.10 | 7.73 ± 0.04 | 7.84 ± 0.05 |
| Dissolved Oxygen (% Sat) | 80.82 ± 4.55 | 83.60 ± 5.22 | 86.76 ± 2.06 | 83.73 ± 2.39 |

Figure 2. Mean percent change in turbidity across treatments.

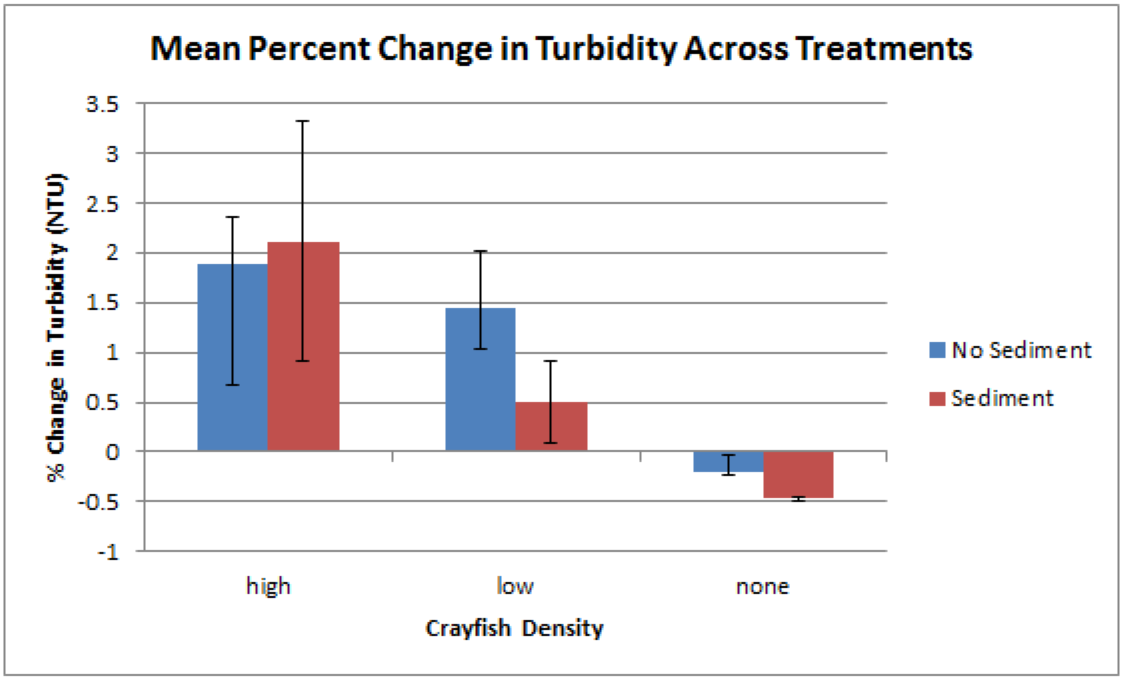


Figure 3. Mean concentration of nitrogen across treatments.

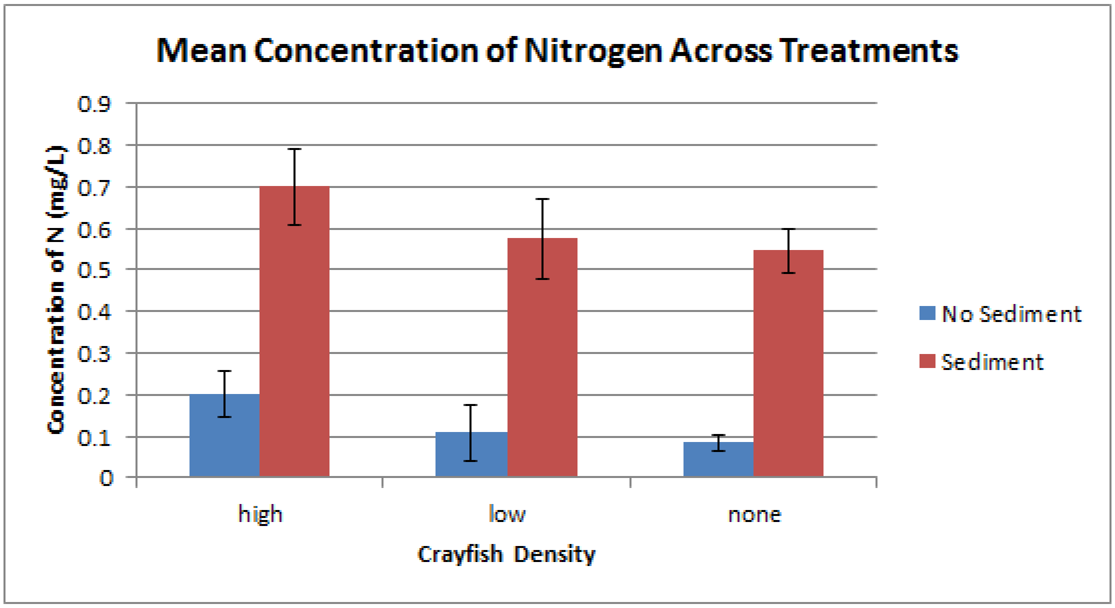


Figure 4. Mean concentration of phosphorus across treatments.